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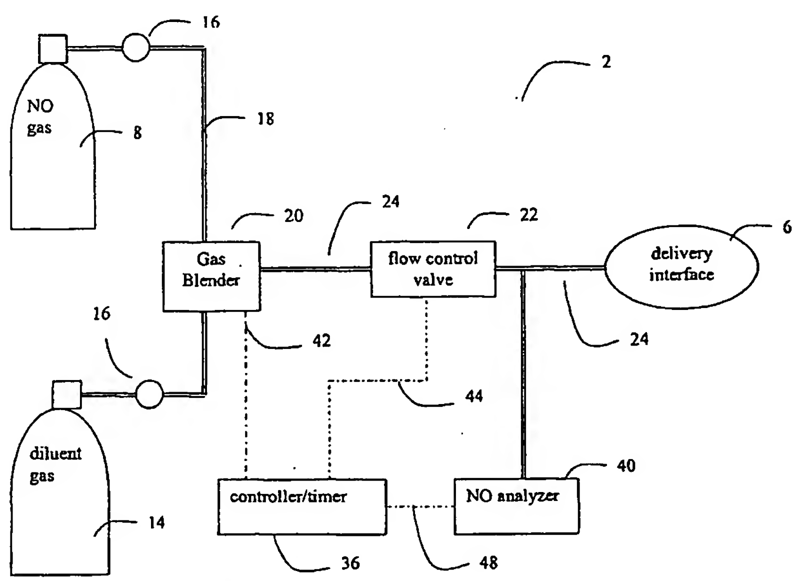
(54) Title: INTERMITTENT DOSING OF NITRIC OXIDE GAS



(57) Abstract: A method and corresponding device are described for combating microbes and infections by delivering intermittent high doses of nitric oxide to a mammal for a period of time and which cycles between high and low concentration of nitric oxide gas. The high concentration of nitric oxide is preferably delivered intermittently for brief periods of time that are interspersed with periods of time with either no nitric oxide delivery or lower concentrations of nitric oxide. The method is advantageous because at higher concentration, nitric oxide gas overwhelms the defense mechanism of pathogens that use the mammalian body to replenish their thiol defense system. A lower dose or concentration of nitric oxide gas delivered in between the bursts of high concentration nitric oxide maintains nitrosative stress pressure on the pathogens and also reduces the risk of toxicity of nitric oxide gas.

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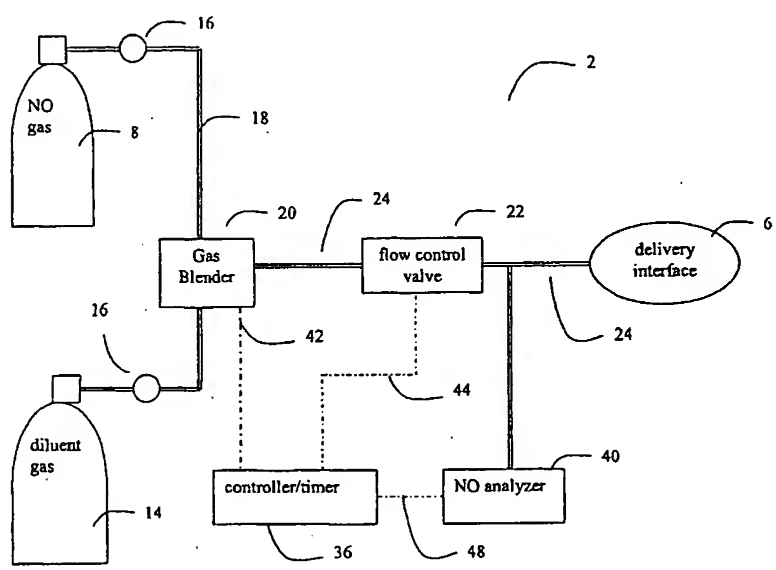

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INTERMITTENT DOSING OF NITRIC OXIDE GAS

FIELD OF THE INVENTION

The field of the present invention relates to methods and devices for delivery of exogenous or gaseous nitric oxide gas to mammals.

BACKGROUND OF THE INVENTION

NO is an environmental pollutant produced as a byproduct of combustion. At extremely high concentrations (generally at or above 1000 ppm), NO is toxic. NO also is a naturally occurring gas that is produced by the endothelium tissue of the respiratory system. In the 1980's, it was discovered by researchers that the endothelium tissue of the human body produced NO, and that NO is an endogenous vasodilator, namely, an agent that widens the internal diameter of blood vessels.

With this discovery, numerous researchers have investigated the use of low concentrations of exogenously inhaled NO to treat various pulmonary diseases in human patients. See e.g., Higenbottam et al., Am. Rev. Resp. Dis. Suppl. 137:107, 1988. It was determined, for example, that primary pulmonary hypertension (PPH) can be treated by inhalation of low concentrations of NO. With respect to pulmonary hypertension, inhaled NO has been found to decrease pulmonary artery pressure (PAP) as well as pulmonary vascular resistance (PVR). The use of inhaled NO for PPH patients was followed by the use of inhaled NO for other respiratory diseases. For example, NO has been investigated for the treatment of patients with increased airway resistance as a result of emphysema, chronic bronchitis, asthma, adult respiratory distress syndrome (ARDS), and chronic obstructive pulmonary disease, (COPD). In 1999, the FDA approved the marketing of nitric oxide gas for use with persistent pulmonary hypertension in term and near term newborns. Because the withdrawal of inhaled nitric oxide from the breathing gas of patients with pulmonary hypertension is known to cause a severe and dangerous

increase in PVR, referred to as a "rebound effect", nitric oxide must be delivered to these patients on a continuous basis.

In addition to its effects on pulmonary vasculature, NO may also be introduced as a anti-microbial agent against pathogens via inhalation or by topical application. *See e.g.*, WO 00/30659, U.S. Patent No. 6,432,077, which are hereby incorporate by reference in their entirety. The application of gaseous nitric oxide to inhibit or kill pathogens is thought to be beneficial given the rise of numerous antibiotic resistant bacteria. For example, patients with pneumonia or tuberculosis may not respond to antibiotics given the rise of antibiotic resistant strains associated with these conditions.

Clinical use of nitric oxide for inhalation has conventionally been limited to low concentration of nitric oxide given the potential toxicity. The toxicity may stem from binding of nitric oxide to hemoglobin that give rise methemoglobin or from the conversion of nitric oxide gas to nitrogen dioxide (NO_2). However, to overwhelm pathogenic defense mechanisms to nitric oxide, it is desirable to deliver nitric oxide at a higher concentration (e.g., between 150 ppm to 250 ppm, and even to 400 ppm) than has traditionally been used clinically for inhalation. Thus, a need exists for a delivery method that is effective against combating pathogens and minimizing the risk of toxicity.

SUMMARY OF THE INVENTION

It is envisioned that a method and device delivering intermittent high doses of nitric oxide for a period of time and which cycles between high and low concentration of nitric oxide is desirable, useful, and overcomes the problems of toxicity. The high concentration of nitric oxide is preferably delivered intermittently for brief periods of time that are interspersed with periods of time with either no nitric oxide delivery or lower concentrations of nitric oxide. This keeps the exposure to the high concentrations

of nitric oxide required to overwhelm the nitric oxide defense mechanisms of the pathogens to an average level that is safe for humans to inhale.

In a preferred embodiment, high concentration of nitric oxide may be delivered at a concentration between 80 ppm to 300 ppm, preferably between 150 ppm to 250 ppm, and more preferably between 160 ppm to 200 ppm. Low concentration of nitric oxide preferably is delivered at a concentration between zero (0) ppm to 80 ppm, and preferably at a concentration of 20 ppm to 40 ppm.

The time periods may vary and in a wide range that preferably will deliver a dose of x time of 600 to 1000 ppmhrs per day. For example, the method would deliver 160 ppm for 30 minutes every four hours with 20 ppm delivered for the 3.5 hours between the higher concentration delivery. High concentration may also be delivered for a period of time between 10 minutes to 45 minutes, and the low concentration is preferably delivered for a period of time longer than the period of time in which the high concentration is delivered. However, it may also be delivered for the same length of time as the high concentration of nitric oxide with less number of cycles to achieve substantially the same amount of ppmhrs of nitric oxide per day. Thus, the high and low concentrations are alternately delivered, and the cycling of the delivery can be for a day, two days, three days, or any other time prescribed by a physician.

Devices for the delivery of nitric oxide are commercially available and may include continuous flow devices, flow matching devices, or pulse dose devices. For example, the FDA has already approved three different nitric oxide delivery systems in the United States: AeroNOx® Delivery System and the ViaNOx DS System (Pulmonox, Canada) and the INOvent® Delivery System (Datex-Ohmeda, Wisconsin). Other devices have also been described in literature and various publications and patents (e.g., U.S. Patent No. 6,581,599, which is incorporated here by reference in its entirety).

In another aspect of the invention, the device for use to deliver intermittent high doses of nitric oxide may include a source of nitric oxide gas (e.g., nitric oxide gas in compressed gas cylinders), controller (e.g. an electronic controller or microprocessor), nitric oxide analyzer, and timer in which the concentration of nitric oxide delivered is automatically changed on a timed basis to a concentration set by the operator and for a set period of time defined by the operator. The device would include logic (e.g. software or firmware) that allows for setting of two different nitric oxide concentrations and with separate time settings for the delivery of each concentration. The device may also include gas mixers (such as gas blenders or combinations of flow control valves and T or Y shaped tube connections), tubings, a source of diluent gas (e.g. room air, oxygen, or inert gas), and electronically regulated needle valves or other valve mechanism for controlling the release of nitric oxide gas, or the diluent gas, or both.

Alternatively, the device may also include two sources of nitric oxide gas, in which one source provides the high concentration of nitric oxide and the other source provides the low concentration of nitric oxide. A switch valve (preferably electronically controlled) is then provided to switch the flow of nitric oxide gas from the high concentration to the low concentration, or vice versa, based on a predefined time. A third source of diluent gas may also be provided to dilute the nitric oxide gas.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-3 illustrate schematic representations of various embodiments of a nitric oxide delivery device according to one aspect of the present invention.

Figure 4 illustrates the logic for setting the alternating delivery profile for high and low concentrations of nitric oxide gas.

Figure 5 illustrates the logic for delivering alternating high and low concentrations of nitric oxide gas.

Figure 6 shows the effect on survival of *S. aureus* (ATCC# 25923) when alternately exposed to NO gas (gNO) exposure at 160 ppm nitric oxide gas for 30 minutes and 20 ppm for 3.5 hours for a total exposure time of 24 hours.

Figure 7 shows the effect on survival of *P. aeruginosa* (ATCC# 27853) when alternately exposed to NO gas (gNO) exposure at 160 ppm nitric oxide gas for 30 minutes and 20 ppm for 3.5 hours for a total exposure time of 24 hours.

Figure 8 shows the effect on survival of *P. aeruginosa* (clinical strain from Cystic Fibrosis) when alternately exposed to NO gas (gNO) exposure at 160 ppm nitric oxide gas for 30 minutes and 20 ppm for 3.5 hours for a total exposure time of 24 hours.

Figure 9 shows the effect on survival of *E. coli* when alternately exposed to NO gas (gNO) at 160 ppm nitric oxide gas for 30 minutes and 20 ppm for 3.5 hours for a total exposure time of 24 hours.

Figure 10 shows the effect on survival of a MshA mycothiol deficient mutant Mycobacterium smegmatis and its wild type counterpart when exposed to 200 ppm NO gas (gNO).

Figure 11 shows the level of mycothiol in wild type *Mycobacterium smegmatis* when exposed to 400 ppm NO gas (gNO) compared to exposure to air.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is currently believed that at higher concentration, nitric oxide gas overwhelms the defense mechanism of pathogens that use the mammalian body to replenish their thiol defense system. The thiol defense system may include for example, the mycothiol for mycobacterium or glutathione for other bacteria. Once this defense mechanism is depleted, the pathogen is defenseless against the killing effects of nitric oxide. A lower dose or concentration of nitric oxide gas delivered in between the bursts of high concentration nitric oxide maintains nitrosative stress pressure on the pathogens to

prevent them from rebuilding their defense system to an adequate level. Thus, a preferred therapeutic or delivery profile for combating pathogens may comprise the delivery of a first concentration of nitric oxide gas for a number of time periods interspersed with intervals in between wherein a second concentration of nitric oxide is administered during the intervals. The first concentration is preferably at a high concentration sufficient to kill or inhibit microbial growth. For example, the first concentration may range from about 80 ppm to 400 ppm, more preferably between 150 to 250 ppm and most preferably between 160 ppm to 200 ppm.

The second concentration is preferably at low concentration of nitric oxide gas such as ranging from 20 to 80 ppm. Alternatively, it should also be understood that the second concentration can also be zero ppm or close to trace amount of nitric oxide gas.

Turning now to the figures, FIGs. 1-3 illustrate various embodiments of a nitric oxide delivery device for use with the present invention. Figure 1 shows, in its most general sense, a NO delivery device 2 that includes a source of nitric oxide gas 8 adapted for delivery of the NO gas to a mammal through a delivery interface 6. Figure 1 illustrates one preferred embodiment of the invention.

In Figure 1, the NO gas source 8 is a pressurized cylinder containing NO gas. While the use of a pressurized cylinder is the preferred method of storing the NO-containing gas source 8, other storage and delivery means, such as a dedicated feed line (wall supply) can also be used. Typically, the NO gas source 8 is a mixture of N_2 and NO. While N_2 is typically used to dilute the concentration of NO within the pressurized cylinder, any inert gas can also be used. When the NO gas source 8 is stored in a pressurized cylinder, it is preferable that the concentration of NO in the pressurized cylinder fall within the range of about 800 ppm to about 10,000 ppm. Commercial nitric oxide manufacturers typically produce nitric oxide mixtures for medical use at around

the 1000 ppm range. Pressurized cylinders containing low concentrations of NO (e.g., less than 100 ppm NO) can also be used in accordance with the device and method disclosed herein. Of course, the lower the concentration of NO used, the more often the pressurized cylinders will need replacement.

Figure 1 also shows a source of diluent gas 14 as part of the NO delivery device 2 that is used to dilute the concentration of NO. The source of diluent gas 14 can contain N_2, O_2, Air, an inert gas, or a mixture of these gases. It is preferable to use a gas such as N_2 or an inert gas to dilute the NO concentration at lower concentration since these gases will not oxidize the NO into NO_2 as would O_2 or air. Nevertheless, for inhalation applications for delivery of high concentration of NO where higher concentration of nitrogen may already be present, the NO flow may be supplemented or diluted with oxygen to prevent the displacement of oxygen by nitrogen that may lead to asphyxiation. It is preferred, especially when delivering higher concentration of NO gas that delivery line downstream of the injection site or gas blender be minimized to reduce the risk of formation of NO_2.

The source of diluent gas 14 is shown as being stored within a pressurized cylinder. While the use of a pressurized cylinder is shown in Figure 1 as the means for storing the source of diluent gas 14, other storage and delivery means, such as a dedicated feed line (wall supply) can also be used. The source of diluent gas can also be a ventilator, air pump, blower, or other mechanical device that moves breathable air.

The NO gas from the NO gas source 8 and the diluent gas from the diluent gas source 14 preferably pass through pressure regulators 16 to reduce the pressure of gas that is admitted to the NO delivery device 2. The respective gas streams pass via tubing 18 to a gas blender 20. The gas blender 20 mixes the NO gas and the diluent gas to produce a NO-containing gas that has a reduced concentration of NO compared to NO

gas contained in the source 8. Preferably, a controller 36 controls the gas blender through electrical connection line 42 such that gas blender can be set to mix the gases to the desired NO concentration (e.g., 160 ppm – 200 ppm for the high concentration period, and 20-40 ppm for the low concentration period) and output via tubing 24.

An optional flow control valve 22 can be located downstream of the gas blender 20 to control the flow of the NO gas to the delivery interface 6. The flow control valve 22 can include, for example, a proportional control valve that opens (or closes) in a progressively increasing (or decreasing if closing) manner. As another example, the flow control valve 22 can include a mass flow controller. The flow control valve 22 controls the flow rate of the NO-containing gas that is input to the delivery device 6.

The delivery interface 6 can be any type of interface adaptable for delivery of the gas to a mammal. For example, if the NO gas is to be delivered to the mammal's airways or lungs, the delivery interface 6 may include a facial mask, nasal insert, or endotracheal tube that interface with the mammal's respiratory system. It should be understood that the types of delivery interface 6 should not be limiting and depends on the specific applications and locations for the delivery of the gas. In another example, if the NO gas is to be delivered topically to a surface of the body such as a skin or eye, a surface of an organ such heart, stomach, etc., a bathing unit as described in U.S. Patent No. 6,432,077, issued to one of the inventors may be used. U.S. Patent No. 6,432,077 is hereby incorporated by reference as if fully set forth herein. Still further example of a delivery interface 6 may an interface to a dialysis circuit or extracorporeal circuitry wherein the NO gas is delivered directly to the blood or body fluids so as to expose the blood or body fluids to NO gas. Such delivery interface are described, for example, in U.S. Patent Application Serial No. 10/658,665, filed on September 9, 2003, which is hereby incorporated by reference in its entirety.

Still referring to Figure 1, the delivery device 2 preferably includes a controller 36 that is capable of controlling the flow control valve 22 and the gas blender 20. The controller 36 is preferably a microprocessor-based controller 36 that is connected to an input device (not shown). The input device may be used by an operator to adjust various parameters of the delivery device such as NO concentration and therapy/exposure time periods. An optional display can also be connected with the controller 36 to display measured parameters and settings such as the set-point NO concentration, the concentration of NO flowing to the delivery interface 6, the concentration of NO_2, the flow rate of gas into the delivery interface 6, the total time of therapy/delivery, and/or the number of cycles for alternating between high and low concentrations of NO gas.

The controller preferably includes a timer for counting down the time periods of the NO gas delivery at the different concentrations. Moreover, the controller preferably includes logic such as firmware or software programs for executing the alternate delivery of high and low concentration of NO gas at pre-set or user programmable time periods. The processes for execution by such logic are illustrated in Figures 4 and 5.

The controller 36 also preferably receives signals through signal line 48 from NO analyzer 40 regarding gas concentrations if such analyzer 40 are present within the delivery device 2. Signal lines 42 and 44 are connected to the gas blender 20 and flow control valve 22 respectively for the delivery and receipt of control signals.

In another embodiment of the nitric oxide delivery device, the controller 36 may be eliminated entirely and the gas blender 20 may be set manually at the desired high or low concentration of nitric oxide gas. The time period may also be tracked manually and at the appropriate set time period, the gas blender is adjusted to either increase to the high concentration NO gas or decrease to the low concentration NO gas. The flow rate of the gas into the delivery interface 6 may be pre-set or adjusted manually.

Figure 2 shows an alternative embodiment of a nitric oxide delivery device 52 in which the desired concentration of NO gas is achieved by mixing with a T or Y shaped connection 70 based on the flow rates of the NO gas flowing from the NO gas source 8 and the diluent gas flowing from the diluent gas source 74. The respective flow rates are controlled via the flow control valves 72 and 75. Mixing of the gases starts at the T or Y shaped connection point 70 and continues through the delivery line 78. An NO analyzer 80 samples the gas mixture at a juncture close to the delivery interface to determine the NO concentration of the gas mixture flowing to the delivery interface 76. The measured NO concentration is then fed back through signal line 88 to the controller 86, which in turn processes the information by comparing the measured NO concentration with the set desired NO gas concentration. The controller 86 then adjusts the flow control valves 72 and 75, if appropriate, by sending control signals through lines 82 and 84 such that the flow rate(s) may be adjusted in order to achieve the desired concentration of NO gas flowing to the delivery interface 76. It should be understood that the controller 86 may similarly include all the features discussed above in connection with controller 36 in Figure 1. Likewise, the delivery interface 76 may be adapted similarly to the delivery interface 6, as described in connection with Figure 1.

Figure 3 illustrates yet another embodiment of a nitric oxide delivery device in accordance to one aspect of the present invention. In this delivery device 102, instead of having gas mixers (e.g., gas blender or T or Y-shaped connection), the delivery device 102 utilizes a switch valve 104 to switch between a high concentration NO gas source 106 and a low concentration NO gas source 108. The switch valve 104 is controlled by the controller 116 that at the appropriate time switches between the high and low concentration of NO gas according to the present invention. It should be understood

that the low concentration NO gas source 108 can also be replaced with non-NO gas source such as air, if the desired period of low NO concentration is zero ppm of NO gas.

Referring now to Figures 4 and 5, process flows are exemplified that may be executed by logic (firmware or software) programmed into the controllers 36, 86, and 116. Figure 4 illustrates a process flow for setting up the desired concentrations and time periods for NO gas delivery starting from Step 400 "START." At Step 405, the logic enters the setup subroutine for setting the desired NO concentrations and time periods. At Step 410, the logic verifies if there are concentration values set for the NO delivery profile. If values are already set, then the process proceeds to Step 415 to verify the values set for the time periods of delivery. If no values have yet been set for the NO concentrations, then the logic calls a subprocess comprising of steps 412 and 414 is called to set the 1st and 2nd NO concentration for the therapeutic profile to be delivered. For example, the 1st NO concentration may be set for about 160 ppm to 300 ppm of NO gas to be delivered and the 2nd concentration may be set for 0 ppm to 80 ppm of NO gas to be delivered. The values of the NO concentrations set are then used by the controller to set the gas blender or the flow control valves in the process illustrated in Figure 5.

After the values of NO concentrations have been set, the logic then proceeds to set the time periods for the delivery of the NO gas in Step 415. If the time periods have not yet been set, then a subprocess comprising steps 417 and 149 is called in which a first time period corresponding to the 1st NO concentration and a 2nd time period corresponding to the NO concentration are set.

After the values of NO concentrations and the time periods have been set, the logic then proceeds to set the number of cycles of alternating 1st and 2nd concentration of NO gas to be delivered. Alternatively, a total therapy time can be set in which the delivery of NO gas will cease at the end of the total therapy time. If the total therapy

time or number of cycles have not been set, then a subprocess comprising of step 422 is called and these values are set. Afterwards, the setup process is ended and the device is ready to deliver NO gas for therapy.

Figure 5 illustrates a process flow for execution by the logic in controller 36, 56, and 116, for the alternating delivery of high and low concentration of NO gas. The START THERAPY in step 500 can be started once the NO gas delivery values in Figure 4 has been entered. At Step 505, the controller 36 (FIG. 1) may then send a control signal through line 42 to the gas blender to set the appropriate gas blender settings to achieve the 1st concentration of NO gas, the value of which was set in the setup process of Figure 4. This process may also include feedback control from the NO analyzer 40 (FIG. 1) to the controller 36 such that the control of the gas blender may be fine tuned in that the actual NO gas concentration being delivered to the delivery interface 6 matches the set NO gas concentration.

Alternatively, the controller at Step 505 may send control signals to the flow control valves 72 and 75 (FIG. 2) to set the appropriate flow rates for the mixing of the gases to achieve the 1st concentration set in the setup process of Figure 4. This process may similarly include feedback control from the NO analyzer 80 (FIG. 2) to the controller 56. In yet another embodiment, the controller at Step 505 may set the switch valve 104 (FIG. 3) to select for delivery the NO gas from a source corresponding to the 1st concentration of NO gas set in the setup process of Figure 4.

Delivery of NO gas proceeds in accordance with the settings in Step 505. At step 510, the timer comprised in the controller 36, 56, or 116 compares the value of the 1st time period set in Figure 4 with the actual countdown in time. If the time period has not elapsed, then the gas blender, flow control valves, or switch valve settings remain the same in Step 512. If the 1st time period has elapsed, then step 515 sets the gas blender,

flow rates, or switch valve settings to that corresponding to the 2nd concentration of NO gas, the value of which was set in the process of Figure 4. Delivery of NO gas then proceeds on the 2nd concentration until the 2nd time period elapsed.

At the completion of the second time period, the logic proceeds to step 525 inquiring into whether the set number of cycles of total therapy time has elapsed. If the set number of cycles or total therapy time has been reached, the therapy ends in Step 530. Otherwise, the process repeats steps 505, 510, 515, and 525.

Further Examples of Delivery Methods

The implementation of the intermittent delivery of high doses of NO gas can be by many means. For example, delivery by inhalation or to the respiratory airway can be made to spontaneously breathing mammals or those managed with mechanical ventilation. With respect to spontaneously breathing mammals, delivery can be achieved via many of previously described gas delivery systems such as masks or nasal cannulas. The device for these mammals may include flowmeter or flow sensor to detect the onset of breathing (e.g., inhalation vs. exhalation) such that the nitric oxide gas would be delivered only when the mammal inhales. Mechanically ventilated mammals would have the nitric oxide delivered into the inspiratory limb of the ventilator circuit and may similarly be triggered only when the ventilator cycled a breath into the mammal.

In both of these implementations, the pattern of nitric oxide delivery may vary depending on the targeted location of the infection within the mammal's lungs and the desire to have the least concentration of nitric oxide residual in the delivery circuit. For example, if the infection were in the air sacs of the lungs, the nitric oxide could be turned off towards the end of the breath when the gas was going to be delivered only to the

airways. As an alternative, if the infection were only in the airways, then the starting gas might have a lower concentration of nitric oxide.

Furthermore, it is preferred that the injection site for NO gas delivery be close to the patient's airway when using higher concentrations of NO gas so as to reduce the time for conversion to NO_2. This minimizes the dwell time of the NO gas in the delivery line before inhalation. Alternatively, the delivery system may utilize a bolus injection of a high concentration at a time point within the breath and allow the dilution of the NO to occur within the lungs.

While embodiments of the present invention have been shown and described, various modifications may be made without departing from the scope of the invention. The invention, therefore, should not be limited, except to the following claims, and their equivalents.

Experimental Results

The effectiveness of the intermittent high dose delivery of nitric oxide gas in combating microorganisms was tested and verified. Briefly, the experimental methods were as follows. Inoculums of varying bacteria was prepared to a suspension of 2.5 x 10^8 cfu/ml, and diluted 1:1000 in sterile normal saline. Three milliliters of the inoculums were used per well in a sterile culture place. Exposure of the inoculums were performed in an exposure chamber, which has been described for example, in Ghafarri, A. et al., "A direct nitric oxide gas delivery system for bacterial and mammalian cell cultures," Nitric Oxide. 12(3):129-40 (May 2005), which is hereby incorporated by reference as if fully set forth herein. The inoculums were exposed to 160 ppm of NO gas at a flow rate of 2.5 liters per minute for 30 minutes followed by exposure to 20 ppm of NO gas for 3.5 hours. The exposure to high and low concentrations of NO gas was repeatedly cycled every 4 hours for 24 hours. At various times (e.g., 0, 4, 8, and 12 hours), samples were

taken and plated to determine the survivability of the bacteria as determined by counting cfu/ml.

Figures 6-9 show the survival of various bacteria used in the experiment with NO gas compared to exposure to air as control. As seen in these figures, cycling exposure to high and low concentrations of nitric oxide is an effective method of killing the bacteria. While it was observed that the effectiveness of cycling exposure to high and low concentrations over a longer period of time, was similar to that of continuous exposure to high concentration, cycling exposure provides a better safety profile in minimizing the risk of methemoglobin formation.

Additional studies were performed to test the hypothesis that the effect of NO gas in killing microorganisms is related to thiol function. Based on studies with various microorganisms, it was observed that Mycobacteria are less sensitive to NO gas damage. This may be due to Mycobacteria having an exceptional thiol, mycothiol, that maintains the redox balance in the cell and protects the cell from nitrosative and oxidative stress. In order to test this hypothesis, sensitivities to NO gas was compared between mycothiol-deficient *Mycobacterium smegmatis* Mutant MshA to its wild type counterpart, mc^2155 by exposing both strains to 200 ppm of NO gas. MshA is an enzyme needed in mycothiol biosynthesis.

Figure 10 shows that the mycothiol-deficient MshA mutant was more sensitive to NO gas than its wild type counterpart and was killed in less time than its wild type counterpart.

Further experiments were conducted to assay and measure the mycothiol level using HPLC in wild type M. smegmatis after exposure to 400 ppm NO gas and were compared to mycothiol level after exposure to air. Figure 11 shows that upon exposure

to 400 ppm of NO gas, the level of mycothiol in the mycobacterium was reduced compared to exposure to air.

Thus, these results show the NO gas may likely act to deplete mycothiol, which is the mechanism by which the mycobacterium protects itself against oxidative stress.

In other bacteria, it is believed that the analogous molecule to mycothiol in mycobacteria is glutathione. The glutathione pool may normally act to protect the bacteria from endogenous NO and H_2O_2, which are released by macrophages against pathogens. Delivery of exogenous NO gas may thus act to overwhelm the glutathione pool, eliminating bacterial protection from H_2O_2, and binding iron based enzymes causing O_2 consumption cessation and electron transport center disruption and freeing metal ions into the bacterial cytosol. The free oxygen, metal ions, NO, and hydrogen peroxide further produce reactive nitrogen and oxygen species as well as metal ions that damage the bacteria's DNA by deamination. Thus, it is believed that cycling or alternating delivery of concentration of NO gas sufficient to overwhelm the glutathione defense mechanism for a period of time and a lower concentration of NO gas may be effective in combating microbes such as bacteria, mycobacteria, and fungi while at the same time exhibit a better safety profile.

Microbes may also include viruses. While viruses do not by themselves have thiol based detoxification pathways, they may still be inherently more susceptible to nitrosative stress. ·NO may inhibit viral ribonucleotide reductase, a necessary constituent enzyme of viral DNA synthesis and therefore inhibit viral replication. Nitric oxide may also inhibit the replication of viruses early during the replication cycle, involving the synthesis of vRNA and mRNA encoding viral proteins. With viruses also depending on host cells for detoxification of the body's defense pathways, the direct cytotoxic mechanisms of NO entering the host cells and the intracellular changes it produces,

could also account for the viricidal effects through viral DNA deamination. Thus, it is believed that the cycling or alternating delivery of NO gas at high and low concentrations may also be effective against viruses.

CLAIMS

1. A method of delivering nitric oxide to a mammal, the method comprising the steps of:

providing a source of nitric oxide gas;

diluting the nitric oxide gas;

alternately administering, for a number of cycles, the nitric oxide gas to the mammal at a first concentration ranging from about 80 ppm to about 400 ppm of nitric oxide gas for a first period of time and at a second concentration of nitric oxide gas lower than the first concentration for a second period of time.

2. The method of claim 1 wherein the second period of time is longer than the first period of time.

3. The method of claim 1 wherein the first concentration of nitric oxide gas ranges from about 160 ppm to about 300 ppm.

4. The method of claim 1 wherein the second concentration of nitric oxide ranges from about 20 ppm to about 40 ppm.

5. The method of claim 1 wherein the first period of time is about 30 minutes and the second period of time is about 3.5 hours.

6. The method of claim 1 wherein the step of administering is through inhalation of the nitric oxide gas.

7. The method of claim 1 wherein the step of administering is topical application of the nitric oxide gas.

8. A method of delivering nitric oxide to mammal, the method comprising the step of administering to a mammal a first concentration of nitric oxide gas for a number of time periods that are interspersed with intervals in between wherein a second concentration of nitric oxide is administered during the intervals.

9. The method of claim 8 wherein the second concentration of nitric oxide gas is lower than the first concentration of nitric oxide gas.

10. The method of claim 9 wherein the second concentration of nitric oxide gas is less than about 80 ppm.

11. The method of claim 8 wherein the first concentration of nitric oxide gas is at a concentration sufficient to kill or inhibit the growth of microbes.

12. The method of claim 11 wherein the microbes are selected from a group consisting of bacteria, mycobacteria, viruses and fungi.

13. The method of claim 8 wherein the step of administration is through inhalation of the nitric oxide gas.

14. The method of claim 8 wherein the step of administering is topical application of the nitric oxide gas.

15. A device for delivery nitric oxide gas comprising:

a source of nitric oxide gas;

a source of diluent gas;

a delivery interface adaptable for delivery of the nitric oxide gas from the source to a mammal;

a gas mixer for mixing the nitric oxide gas with the diluent gas;

a controller that communicates with the gas mixer wherein the controller comprises logic for setting a nitric oxide delivery profile comprising at least two different concentrations of nitric oxide gas and for automatically switching between the at least two different concentrations of nitric oxide gas on a timed basis.

16. The device of claim 15 wherein the delivery profile further comprises at least a first and a second time period corresponding respectively to each of the at least two different concentration of nitric oxide gas.

17. The device of claim 16 wherein the first time period is shorter than the second time period.

18. The device of claim 15 wherein the gas mixer comprises a T or Y shaped tubing connection and a flow control valve.

19. The device of claim 15 wherein the gas mixer comprises a gas blender.

20. The device of claim 15 wherein the delivery interface comprises a bathing unit for topical delivery of nitric oxide gas to a surface of the body.

21. The device of claim 15 wherein the delivery interface comprises an interface selected from a group consisting of facial mask, nasal insert, and endotracheal tube.

22. The device of claim 15 further comprising a nitric oxide gas analyzer for measuring the concentration of nitric oxide gas flowing to the delivery interface, wherein the nitric oxide gas analyzer sends signals to the controller.

23. A device for delivery nitric oxide gas comprising:

a source of nitric oxide gas at a first concentration;

a source of breathable gas;

a delivery interface adaptable for delivery of the nitric oxide gas from the source to a mammal;

a switch valve downstream of the source of nitric oxide gas and upstream of the delivery interface, said switch valve for directing the flow of nitric oxide gas from the source to the delivery interface;

a controller controlling the switch valve and which commands the switch valve to switch between the source of nitric oxide gas and the source of breathable gas on a timed basis.

24. The delivery device of claim 23 wherein the source of breathable gas comprises nitric oxide gas at a concentration lower than the first concentration of nitric oxide gas.

FIG. 1

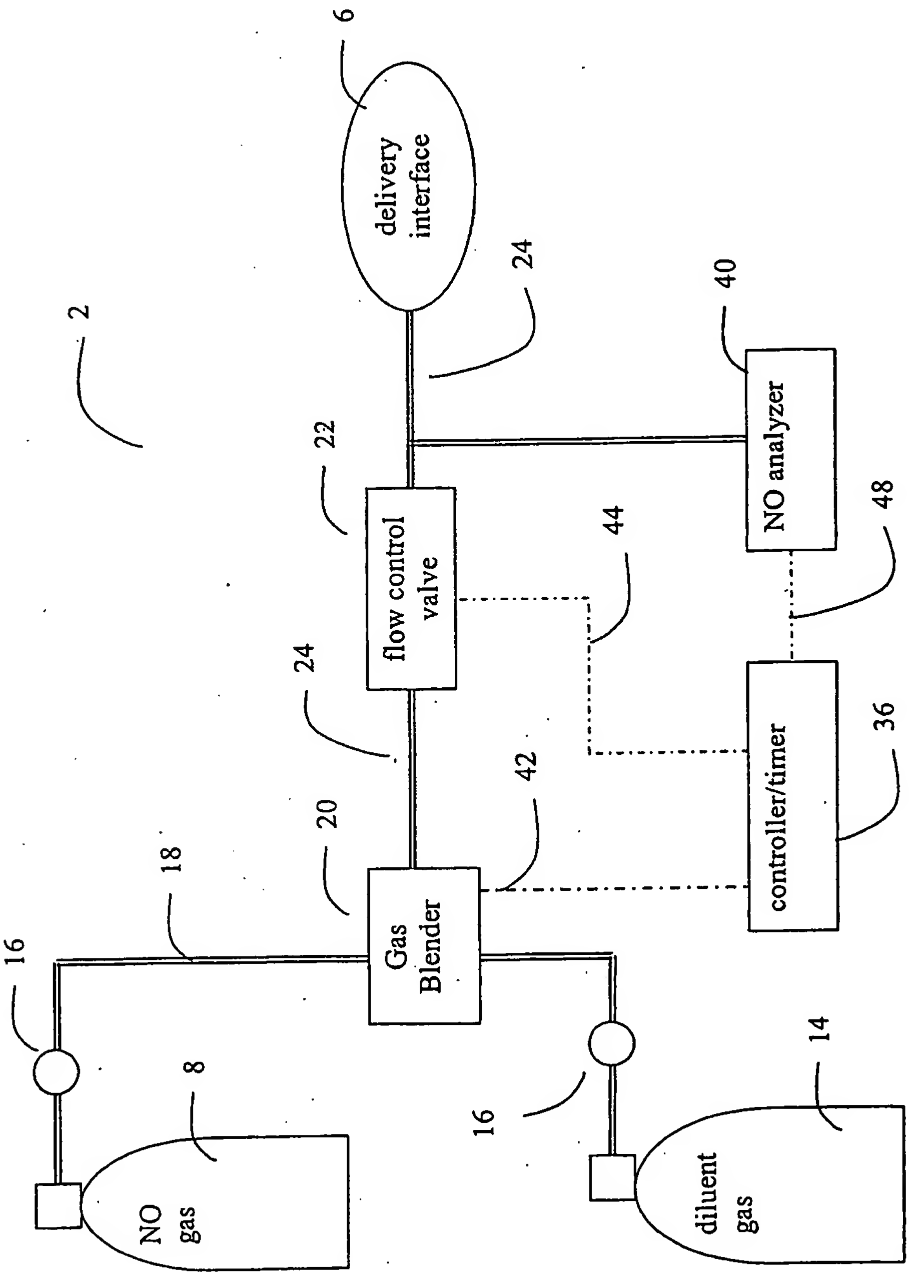
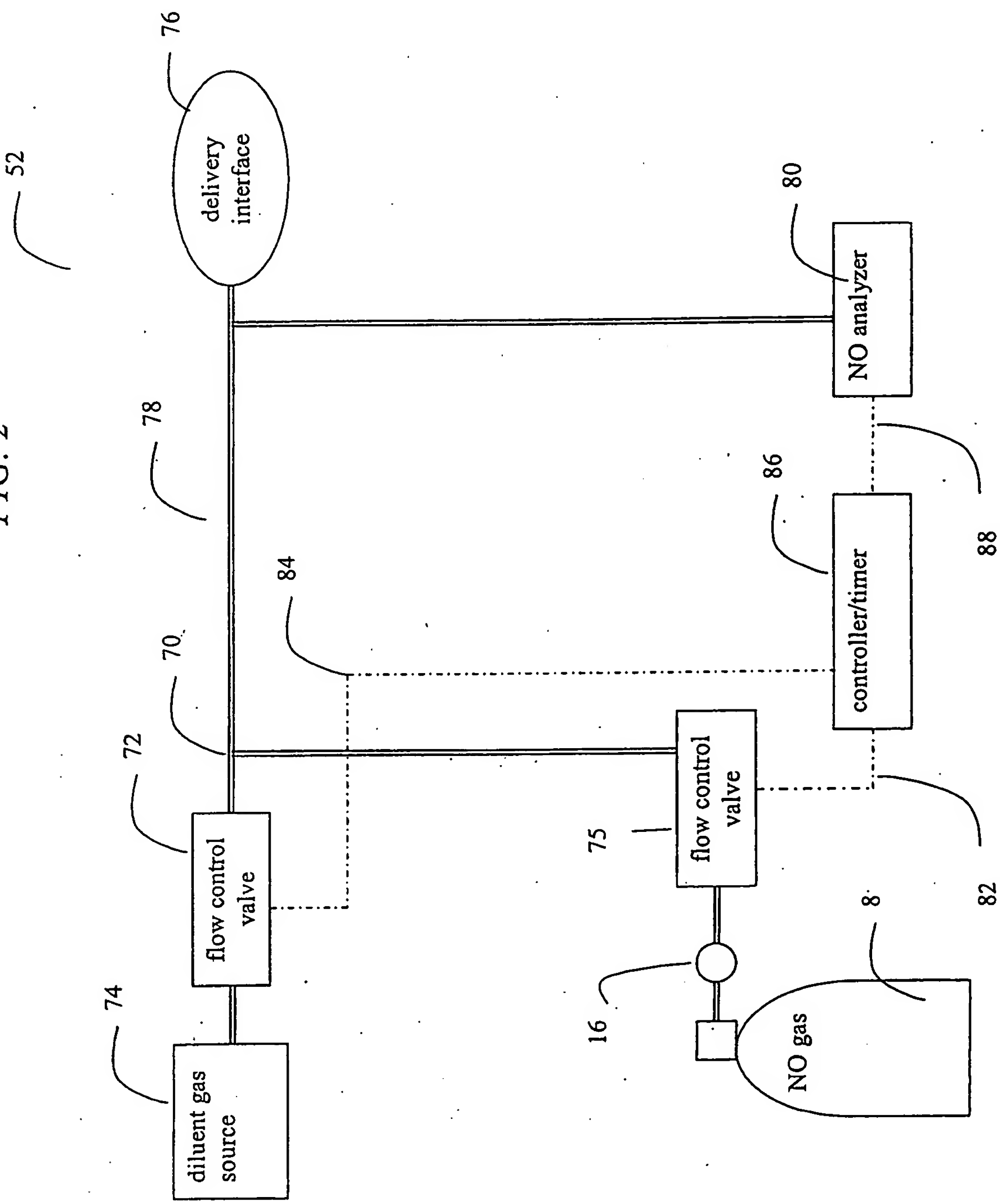

2
6
delivery interface
24
40
NO analyzer
22
flow control valve
48
44
24
controller/timer
36
42
20
Gas Blender
18
16
8
NO gas
16
14
diluent gas

FIG. 2


52
76
delivery interface
80
NO analyzer
78
86
controller/timer
88
84
70
72
flow control valve
75
flow control valve
82
74
diluent gas source
16
8
NO gas

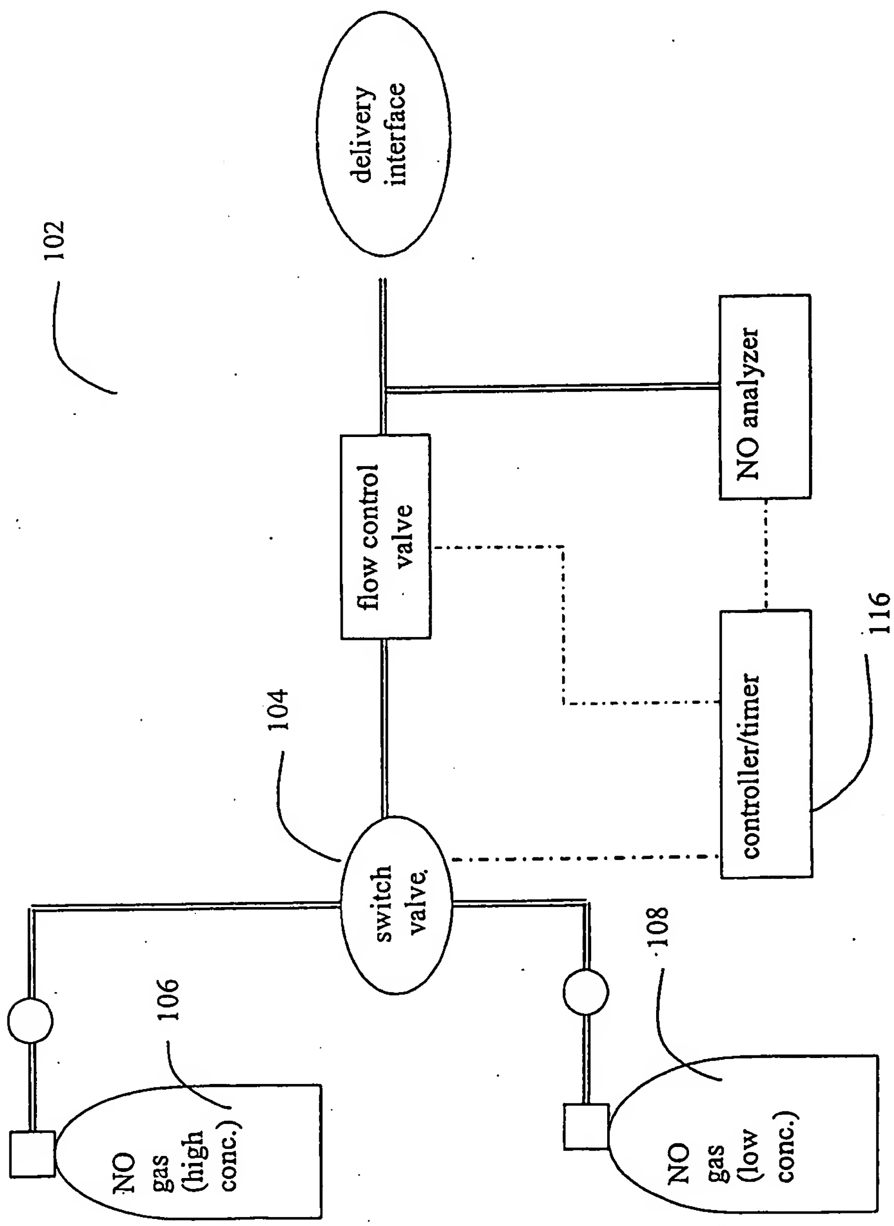

102
delivery interface
NO analyzer
flow control valve
controller/timer
116
104
switch valve
108
106
NO gas (high conc.)
NO gas (low conc.)

FIG. 3

FIG. 4

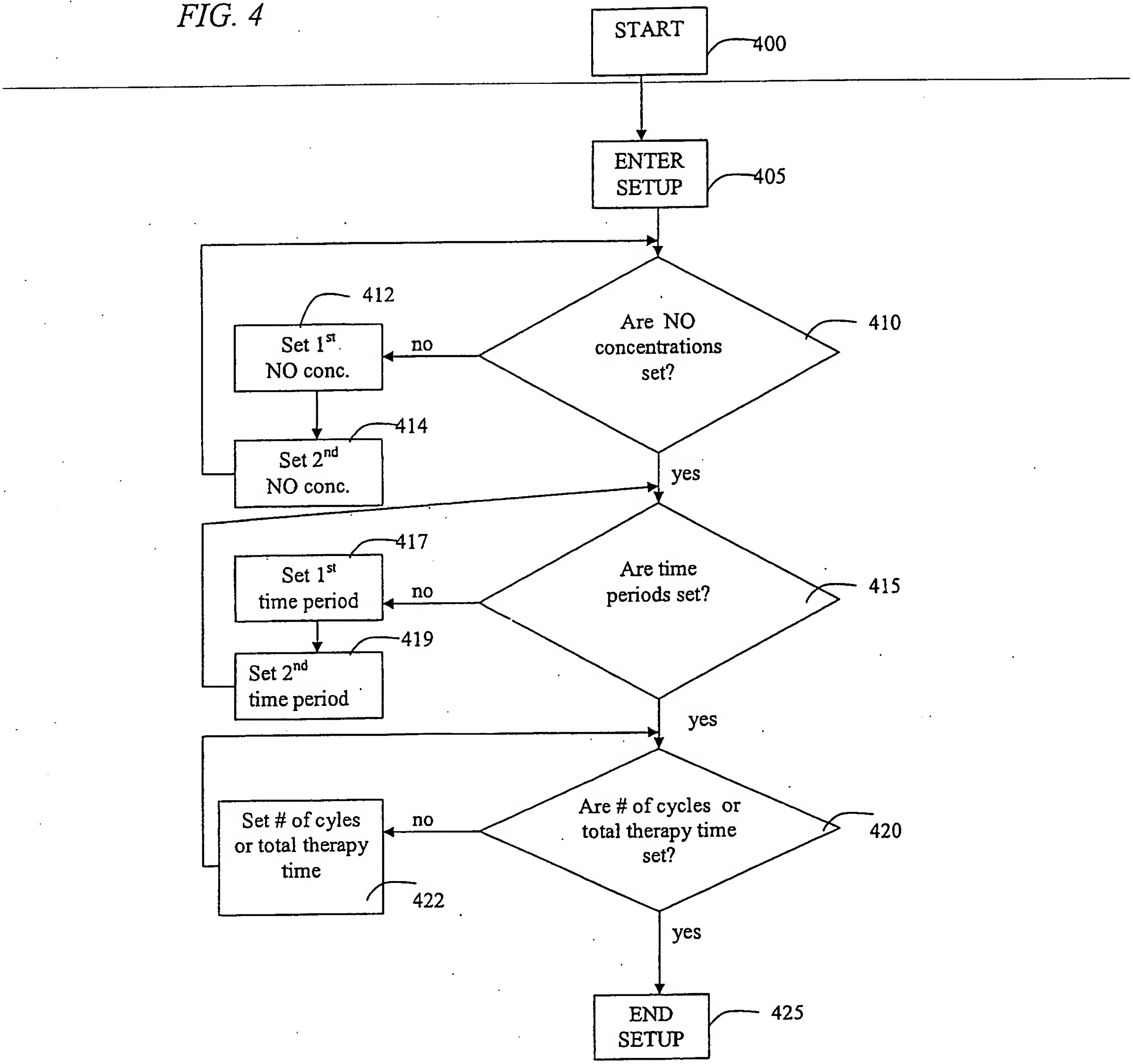

START
400
ENTER SETUP
405
Are NO concentrations set?
410
no
Set 1st NO conc.
412
Set 2nd NO conc.
414
yes
Are time periods set?
415
no
Set 1st time period
417
Set 2nd time period
419
yes
Are # of cycles or total therapy time set?
420
no
Set # of cyles or total therapy time
422
yes
END SETUP
425

FIG. 5

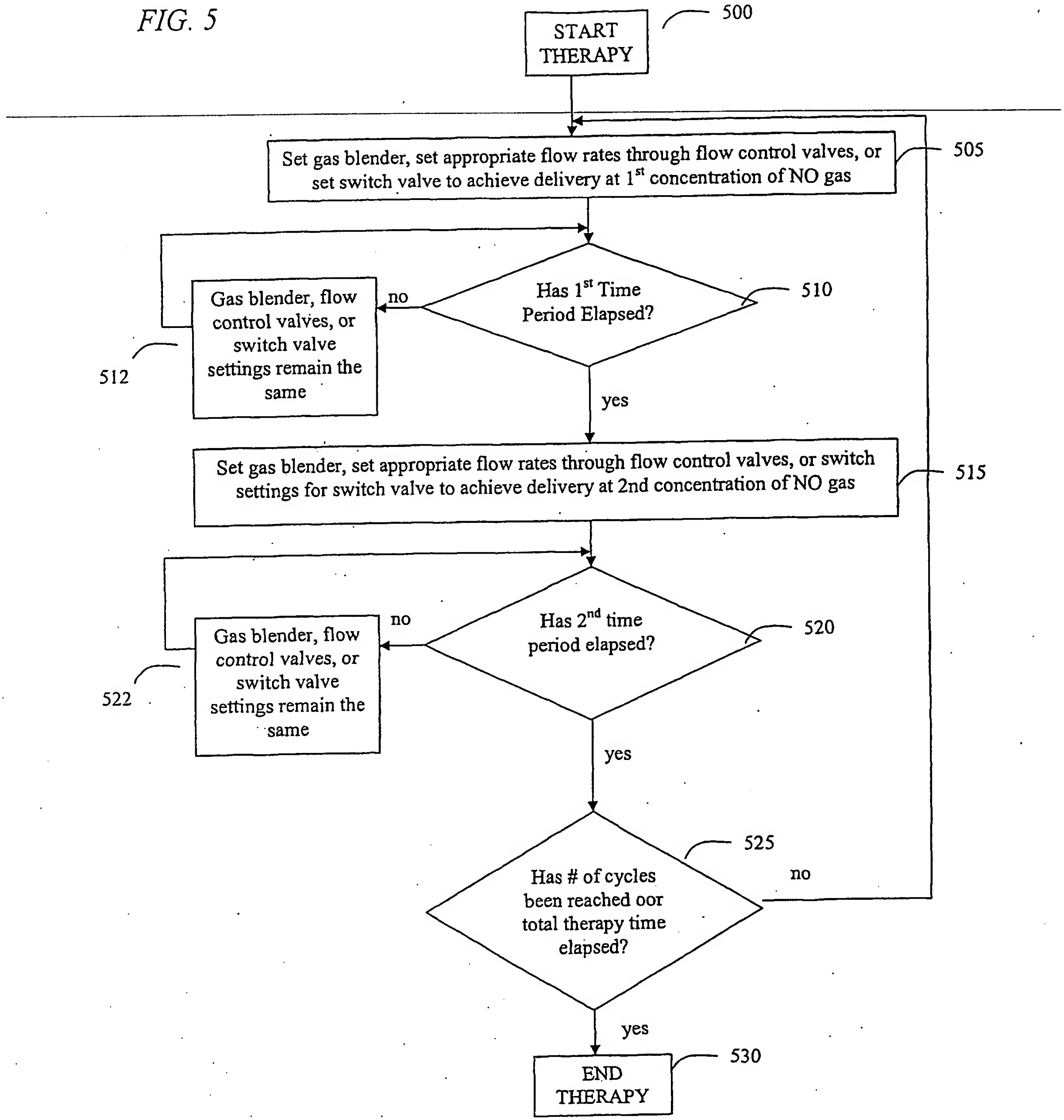

START THERAPY
500
Set gas blender, set appropriate flow rates through flow control valves, or set switch valve to achieve delivery at 1st concentration of NO gas
505
Has 1st Time Period Elapsed?
510
no
Gas blender, flow control valves, or switch valve settings remain the same
512
yes
Set gas blender, set appropriate flow rates through flow control valves, or switch settings for switch valve to achieve delivery at 2nd concentration of NO gas
515
Has 2nd time period elapsed?
520
no
Gas blender, flow control valves, or switch valve settings remain the same
522
yes
Has # of cycles been reached oor total therapy time elapsed?
525
no
yes
END THERAPY
530

Fig. 6

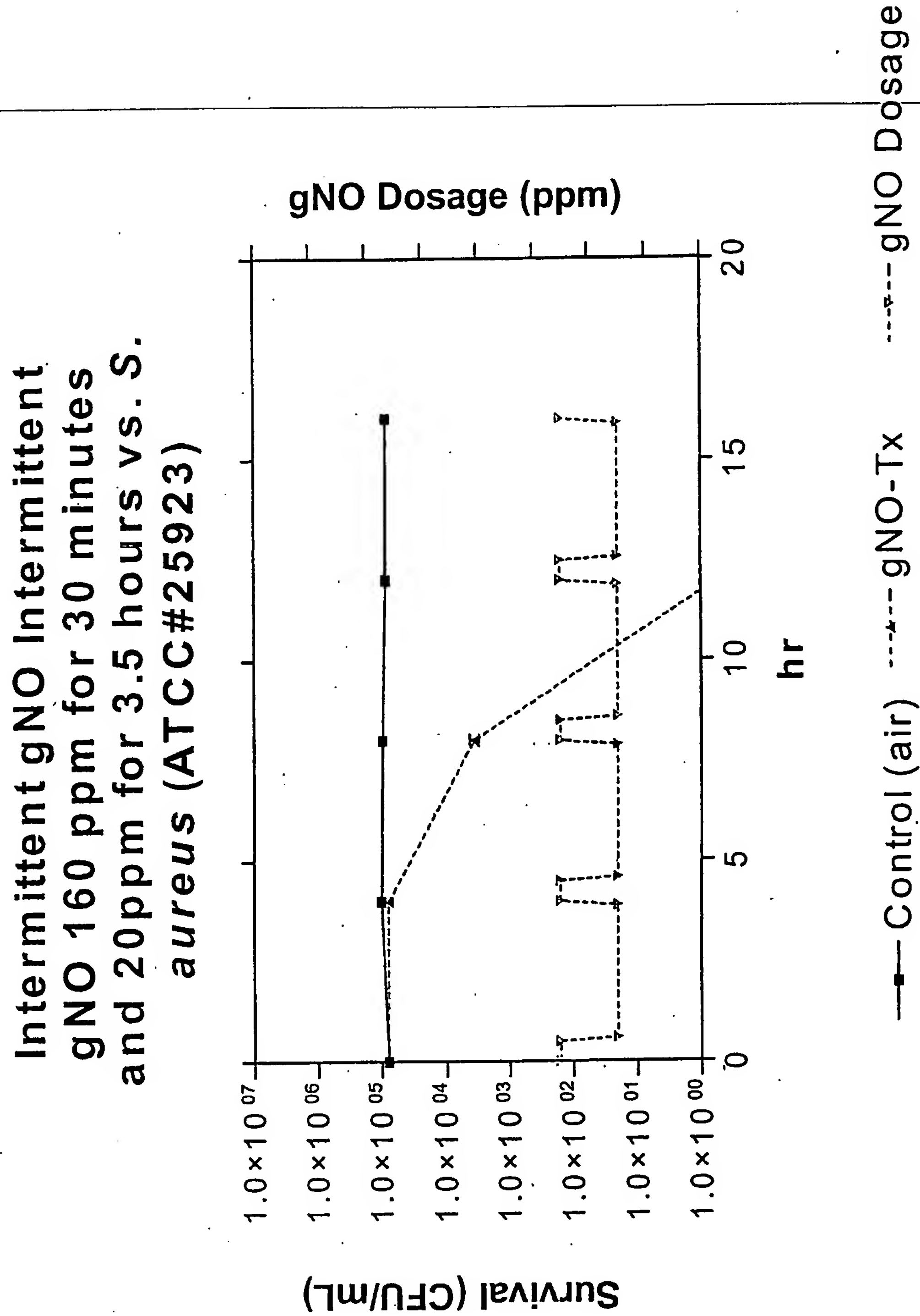

Intermittent gNO Intermittent gNO 160 ppm for 30 minutes and 20ppm for 3.5 hours vs. S. aureus (ATCC#25923)
gNO Dosage (ppm)
Survival (CFU/mL)
1.0×10 07
1.0×10 06
1.0×10 05
1.0×10 04
1.0×10 03
1.0×10 02
1.0×10 01
1.0×10 00
0
5
10
15
20
hr
Control (air)
gNO-Tx
gNO Dosage

Fig. 7

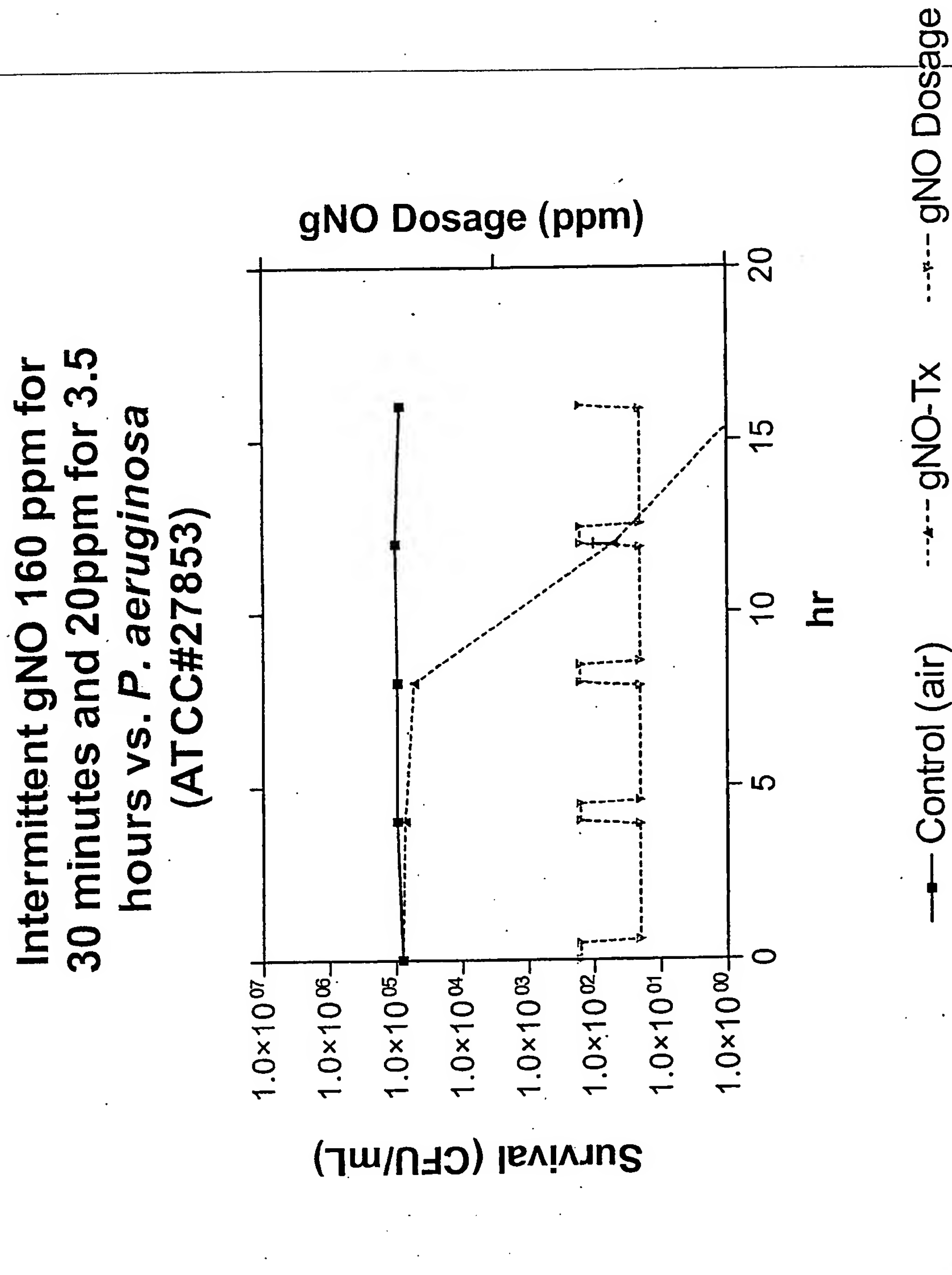

Intermittent gNO 160 ppm for 30 minutes and 20ppm for 3.5 hours vs. P. aeruginosa (ATCC#27853)
gNO Dosage (ppm)
Survival (CFU/mL)
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
0
5
10
15
20
hr
Control (air)
gNO-Tx
gNO Dosage

Fig. 8

Intermittent gNO 160 ppm for 30 minutes and 20ppm for 3.5 hours vs. *P. aeruginosa* (Clinical Cystic Fibrosis)

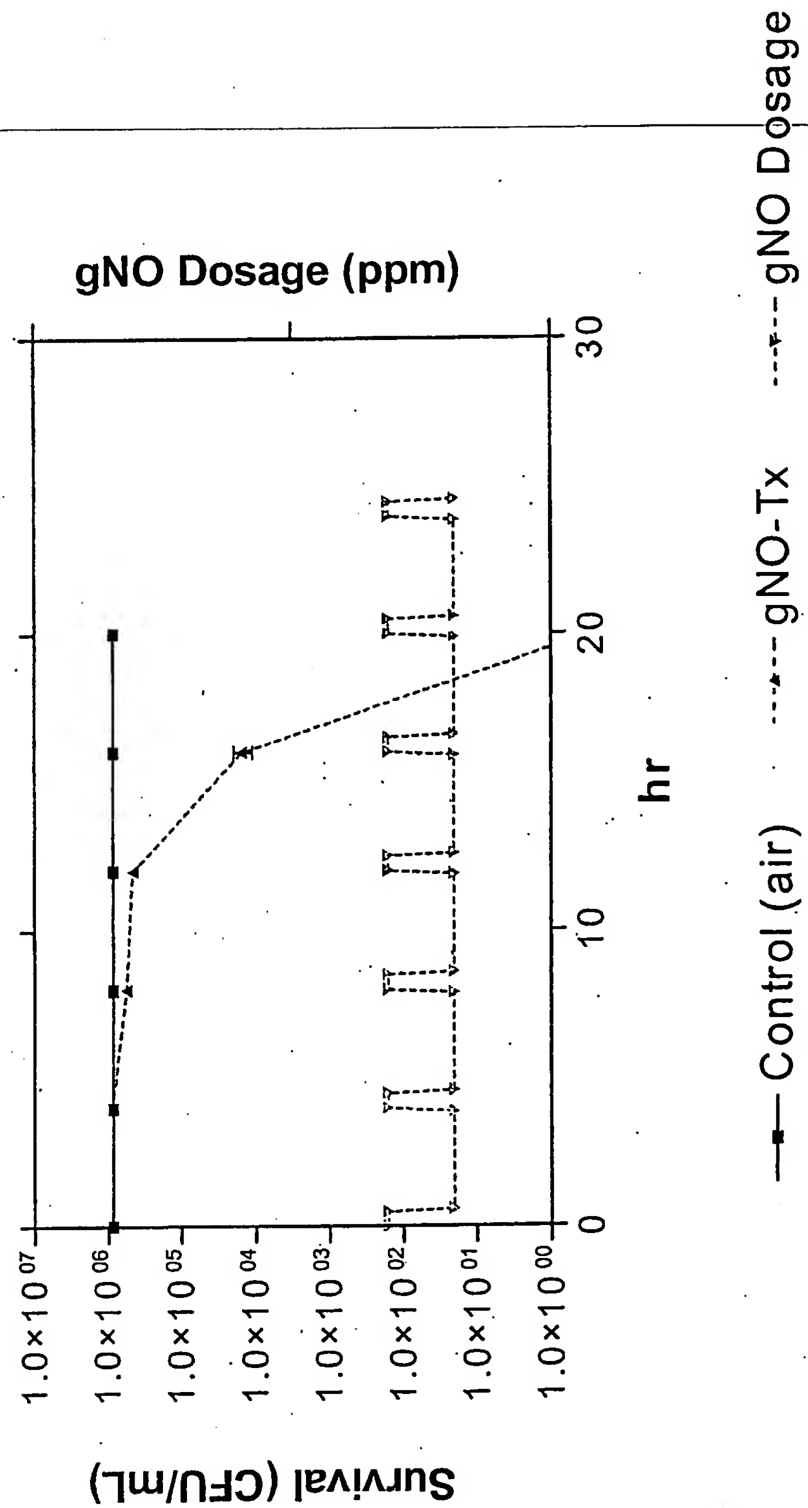


Fig. 9

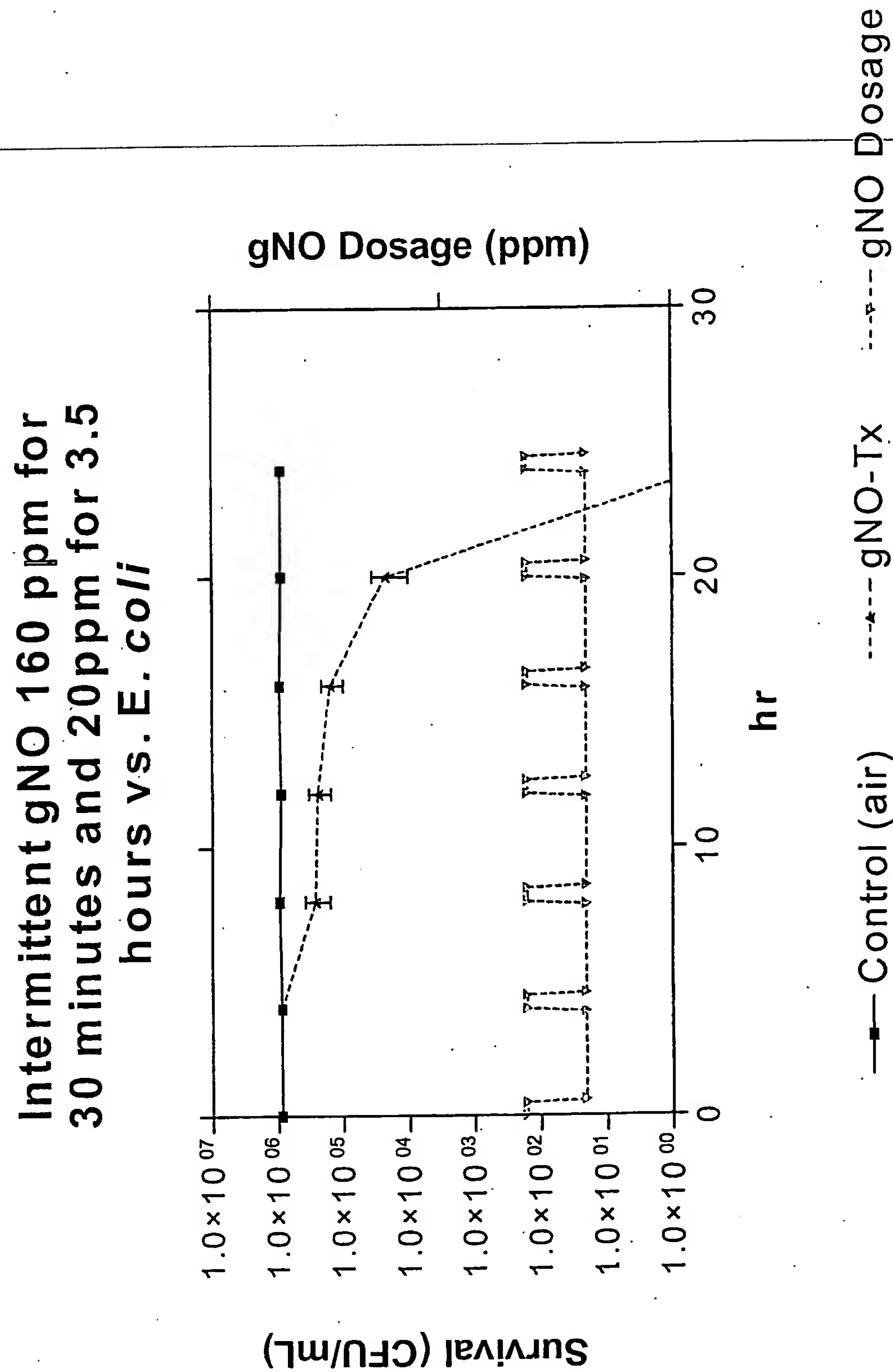

Intermittent gNO 160 ppm for 30 minutes and 20ppm for 3.5 hours vs. E. coli
gNO Dosage (ppm)
Survival (CFU/mL)
1.0×10 07
1.0×10 06
1.0×10 05
1.0×10 04
1.0×10 03
1.0×10 02
1.0×10 01
1.0×10 00
0
10
20
30
hr
Control (air)
gNO-Tx
gNO Dosage

Fig. 10

Effect of 200ppm gNO on Wild Type and MshA Mycothiol deficient Mutant *Mycobacterium smegmatis*

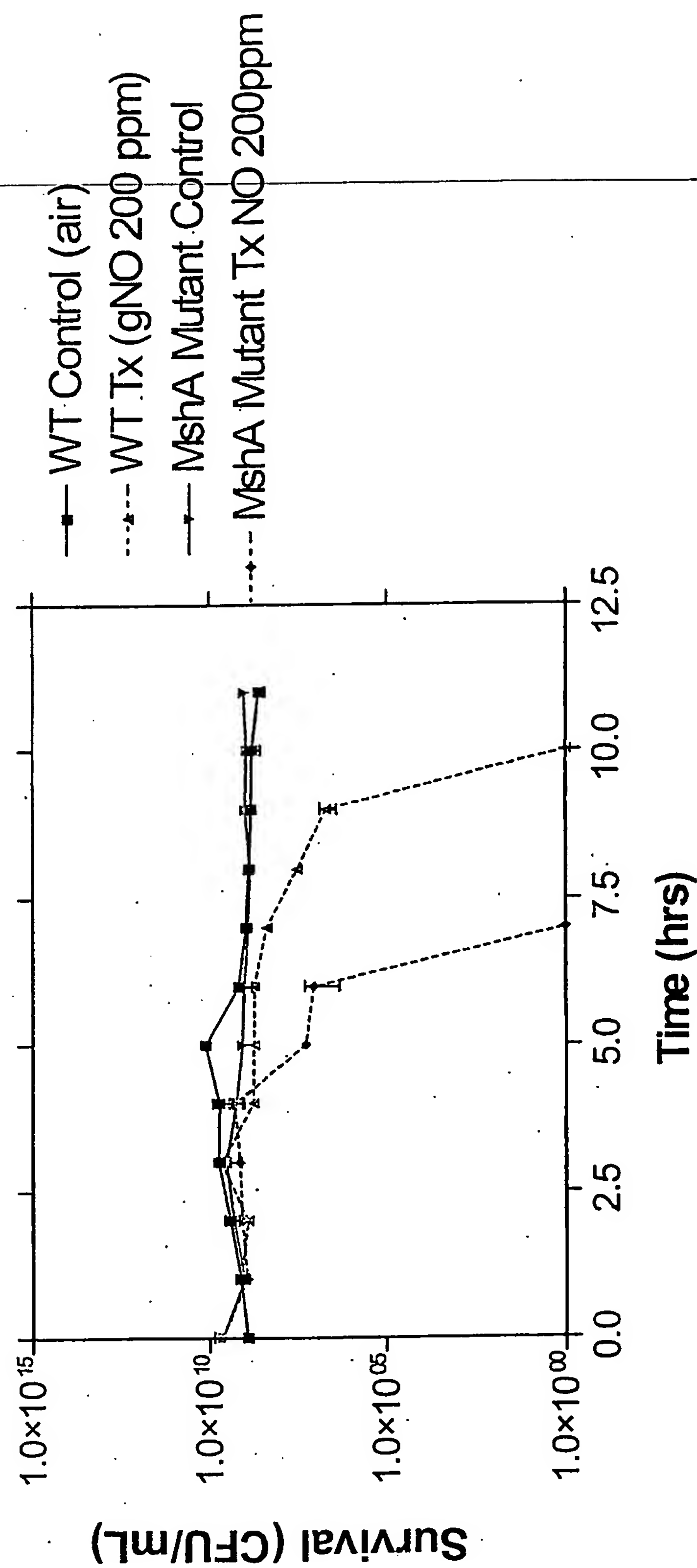
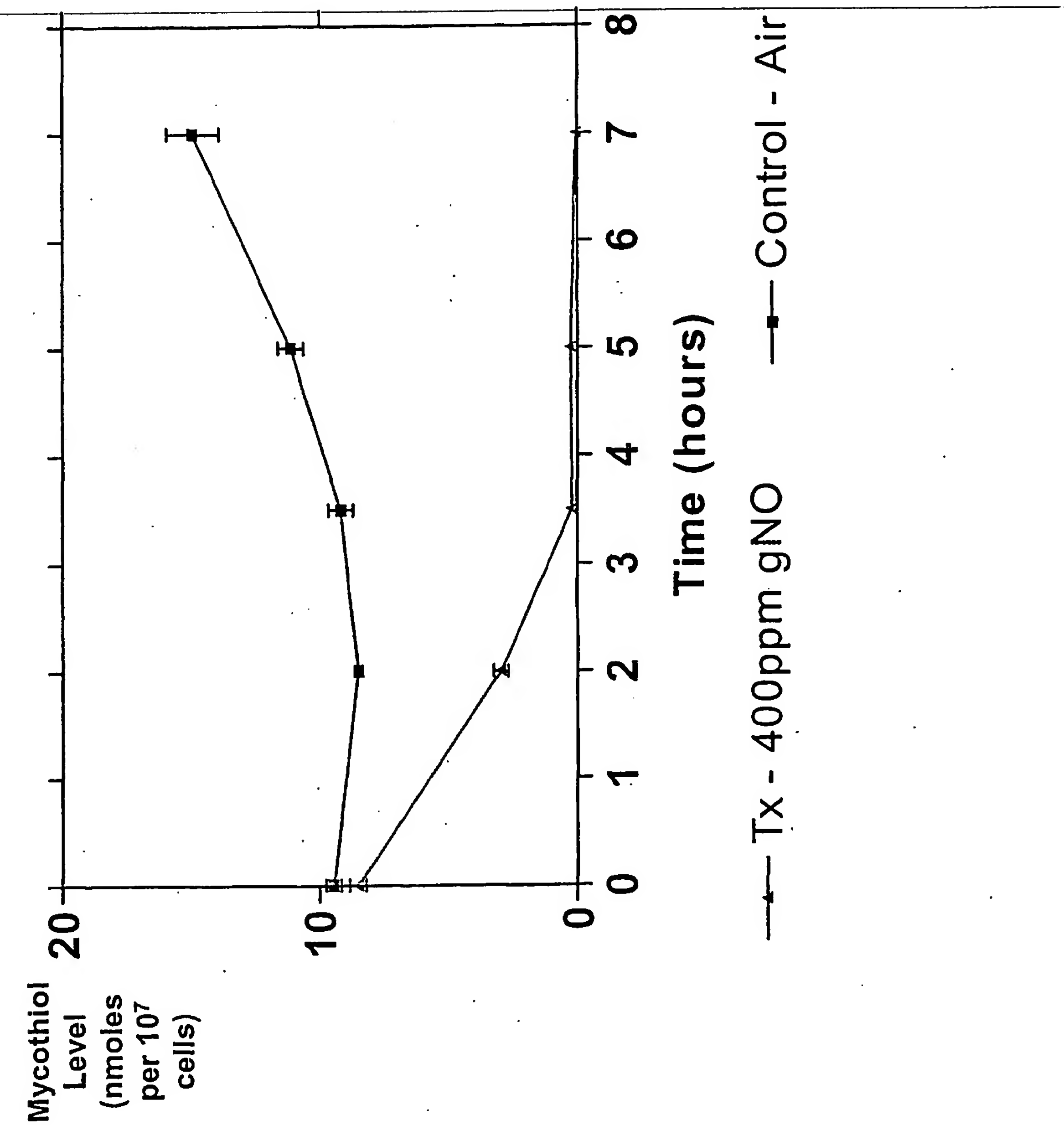


Fig. 11


Mycothiol Level (nmoles per 10^7 cells)
20
10
0
0
1
2
3
4
5
6
7
8
Time (hours)
Tx - 400ppm gNO
Control - Air